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# Renal kallikrein-kinin: Its relation to renal prostaglandins and renin-angiotensin-aldosterone in man

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Several vasoactive substances are generated in the kidney. One is kallikrein, an enzyme generated in the renal cortex that catalyzes the formation of the vasodilator hormone lysylbradykinin (kallidin) from plasma alpha-2 globulin substrate, kininogen. Urinary kallikrein and kinin appear to be derived from the kidney and differ from those in circulating plasma. In our previous study [1], the intrarenal arterial infusion of synthetic bradykinin did not cause any increase in kinin excretion in urine collected directly from the ipsilateral ureter in dogs, in spite of the occurrence of a prominent diuresis and natriuresis. This suggests that circulating plasma kinin filtered through the glomeruli might be rapidly destroyed by kininase II in the proximal tubule and that kinin excreted in the urine might originate from the renal tubules. Nustad, studying the distribution of kallikrein in the rat renal tissue, found almost all the activity to be in the renal cortex, whereas he found only a little activity in the medulla [2]. A similar result was obtained in the dog by Carretero and Scicli [3]. Their study with the stop-flow method has shown that renal kallikrein is secreted into the tubular lumen at the level of the distal tubule. This observation is in agreement with the study [4] on the immunohistochemical localization of kallikrein in the same part of the renal tubule that is juxtaposed to the enzyme renin, located in the wall of the glomerular afferent arteriole. From these results, it is reasonable to presume that a major site of kinin generation is in the distal tubule and that the generated kinin is secreted into the urine at this site. Now, it is generally accepted that the urinary excretion of kallikrein and kinin reflects the generation and/or the release of renal kallikrein and kinin from the kidney.

The renal medulla and papilla are a rich source of prostaglandin (PG) synthetase [5]. Since the discovery and identification of $PGE_2$, $PGA_2$, and $PGF_{2\alpha}$ from the rabbit renal medulla by Lee et al [6], many studies have been done. The observations that $PGE_2$ and $PGA_2$ not only lowered blood pressure but also had notable effects on enhancing renal blood flow, diuresis, and natriuresis, and that $PGA_2$, unlike $PGE_2$, was not degraded in its passage across the pulmonary circulation [7] suggested that $PGA_2$ might be a circulating hormone. But, there is no evidence that $PGA_2$ is a normal enzymatic product in the kidney. According to Larsson and Änggard [8], $PGA_2$ was not found in amounts exceeding 10% of the $PGE_2$ content in the rabbit renal medulla, analyzed by gas chromatographic-mass spectrometric determination. Thus, the major PG's in the renal medulla are $PGE_2$ and $PGF_{2\alpha}$. $PGE_2$ has a very short half-life in blood, because it is removed almost completely by a single passage through the pulmonary circulation. Therefore, it is very difficult to evaluate the pathophysiologic roles of renal $PGE_2$ by estimating its concentration in peripheral blood. Frölich et al [9] have shown, however, that renomedullary PG's are excreted in urine and that their excretion rates reflect the synthesis or the release of renal PG's.

Previous animal experiments have shown that there are complicated interactions between renal kallikrein-kinin and renin-angiotensin-aldosterone or renal kallikrein-kinin and renal PG's. The observations by Erdös and Yang [10] that the kidney has a very rich kininase content and that kininase II, located in proximal tubule, is identical with the angiotensin I converting enzyme linked the renal

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kallikrein-kinin system to the renin-angiotensin system. There are several reports indicating another linkage of these two systems. Geller et al [11] showed that the urinary excretion of kallikrein was increased after the administration of deoxycorticosterone acetate (DOCA) in rats, but that it was decreased after bilateral adrenalectomy. Margolius, Chao, and Kaizu [12] also reported that the release of kallikrein from a rat renal cortical cell suspension was increased after the addition of aldosterone and decreased after the addition of the aldosterone antagonist, spironolactone, indicating that the generation or the release of renal kallikrein is regulated by aldosterone or some other sodium-retaining steroid hormones. Mills et al [13] found that the urinary excretion of kallikrein was increased after the intrarenal arterial infusion of angiotensin II (AII) in the dog, suggesting that AII may directly contribute to the regulation of renal kallikrein release. These reports strongly support the existence of an interaction between renal depressor substances (the kallikrein-kinin system) and adrenorenal pressor substances (the renin-angiotensin-aldosterone system).

On the other hand, McGiff, Itskovitz, and Terragno [14] reported that the intrarenal infusion of bradykinin stimulates the release of a PGE-like substance from the dog kidney, with an enhancement of renal blood flow and water and sodium excretion. This enhancement of the PG release and the renal vasodilator action of kinin was prominently attenuated after the inhibition of endogenous PG generation by indomethacin, suggesting that there is a coupling between the kinin and the PG systems within the kidney. Kinins increase PG synthesis, and the PG, in turn, modifies the effects of kinin on the renal hemodynamics and excretory function. Recently, Nasjletti, McGiff, and Colina-Chourio [15] reported that the administration of DOCA or aldosterone increased the urinary excretion of kallikrein and PGE and produced polyuria but did not affect sodium excretion, suggesting that interactions of mineralocorticoid hormones, kinins, and PG's may be important in the maintenance of salt-water homeostasis.

There is also close interaction between the renal PG's and the renin-angiotensin system. According to the reports by McGiff et al [16], the intrarenal arterial infusion of AII stimulated the release of PG from the kidney, counteracting the vasoconstricting effect of AII. On the other hand, Larsson, Weber, and Änggard [17] reported that the enhanced PG synthesis induced by the administration of the PG precursor, arachidonic acid, raised the plasma renin activity, whereas inhibition of PG synthesis with indomethacin decreased plasma renin activity, suggesting that the renal PG system may contribute to the renin release mechanism.

Previous reports concerning the roles of renal kallikrein-kinin and renal PG's in renal sodium handling have been conflicting. The intrarenal infusion of kallikrein-kinin and also of PGE results in a prominent natriuresis and diuresis, indicating that renal kallikrein-kinin and renal PGE may be involved in the regulation of renal sodium excretion [18, 19]. Marin-Grez, Cottone, and Carretero [20] found that kinins in blood obtained from the inferior vena cava and urinary kallikrein excretion were increased after acute intragastric salt loading in the dog, whereas blood kinins were not increased in bilaterally nephrectomized, sodium-loaded dogs. In contrast to sodium loading, kallikrein excretion was less in the urine of water-loaded dogs than it was in sodium-loaded dogs, and no change in the circulating kinins was observed in the dogs with water-loading. Their results suggest that the increase of plasma kinin after acute sodium loading depends on the presence of the kidney and that the activated kallikrein-kinin might be contributing to the mechanism of sodium handling. Marin-Grez, Oza, and Carretero [21] also reported that urinary kallikrein was increased in dogs in a state of escape from the sodium-retaining effect of DOCA. On the contrary, there are many reports stating that urinary excretion of kallikrein was decreased during a high sodium diet and increased during a low sodium diet in animals as well as in man [22–25].

Studying the roles of renal PGE in renal sodium handling, Tobian and O'Donnell [26] found that the renal PGE content in the rat was decreased after sodium loading, which suggests that renal PGE may act as an antinatriuretic hormone. In contrast, our previous reports [25, 27] showed that there was a significant positive correlation between urinary PGE excretion and urinary sodium excretion in normal volunteers and in patients with essential hypertension, suggesting that renal PGE may contribute to the renal sodium excretion. As shown [25], there are complicated regulatory mechanisms in the release of renal kallikrein and also complicated interactions among the renal vasoactive substances.

The present study was done to investigate the physiologic regulatory mechanism of generation and/or release of renal kallikrein and kinin in relation to the renin-angiotensin-aldosterone system and renal PGE in man by measuring the urinary

excretion of kallikrein, kinin, $PGE_2$, and $PGF_{2\alpha}$, plasma renin activity (PRA), and plasma aldosterone concentration (PAC) under physiologic condition. The renin-angiotensin-aldosterone system was stimulated with a low sodium diet and furosemide injection, and the system was suppressed with a high sodium diet and the aldosterone antagonist spironolactone. Endogenous PG generation was suppressed by indomethacin. Acute plasma volume expansion was produced with saline infusion or water loading.

## Methods

*Urinary kinin.* Urinary kinin was measured by the method of Carretero et al [28] and Abe et al [29]. Collected urine was stored immediately at −20° C until the assay. The radioimmunoassay was performed in 0.1 M Tris hydrochloric acid buffer (pH, 7.4) containing 0.2% of gelatin and 0.1% of neomycin (buffer A). The incubation system consisted of $^{125}$I-8-tyrosine-bradykinin, 3000 cpm (specific radiologic activity, 800 to 1000 mCi/μM; Daiichi Radioisotope Corp.), urine (0.01 to 0.05 ml), and antiserum (0.1 ml; 1:16,000), adjusted to a final volume of 0.8 ml with buffer A. The mixture was incubated for 24 hours at 4° C, and free kinin was separated with dextran-coated charcoal. After the radioactivity was counted, the kinin content was calculated. This method is sensitive down to 10 pg of kallidin. The recovery rate of added kallidin (50 to 500 pg) was 97 ± 4% (mean ± SEM, $N = 15$). The metabolic fragments produced by incubating bradykinin, kallidin, and methionyl-lysyl-bradykinin with chymotrypsin showed a 0.5% crossreaction with kinin antiserum. The values of urinary kinin determined by the present method in 32 subjects showed a highly significant correlation with the values determined by a bioassay consisting of extraction and assay using the autoperfused dog femoral arterial blood flow ($r = 0.71$, $P < 0.001$).

*Urinary kallikrein.* Urinary kallikrein activity was measured as the kininogenase activity by the radioimmunoassay of generated kinin [29]. Urine (0.05 to 0.1 ml) was incubated with 4 μg of low-molecular-weight kininogen (supplied by Dr. Kato, Protein Research Institute, Osaka) dissolved in 0.4 ml of 0.1 M phosphate buffer (pH, 8.4) containing 0.1% neomycin, 3 mM 8-hydroxyquinoline, and 30 mM disodium ethylenediaminetetraacetic acid (EDTA) at 37° C for 20 min. After the incubation, the mixture was diluted with distilled water, heated at 80° C for 15 min to stop the enzymatic reaction, and stored at −20° C until the measurement. The kinin generated during the incubation was determined radioimmunologically. In the present method, the extraction procedure was not necessary, because low-molecular-weight kininogen had no crossreaction to the antiserum. The values of urinary kallikrein determined by the radioimmunoassay in 31 subjects showed a highly significant correlation with the values determined by the esterolytic activity of *p*-tosyl-arginine-methyl-ester (TAME) ($r = 0.78$, $P < 0.001$).

*Urinary prostaglandin.* Urinary PGE was measured radioimmunologically [30]. PGE in urine (3 to 5 ml) was converted to PGB by alkaline treatment according to Zusman's method [31]. Then, the sample was acidified to a pH of 3 to 4 with hydrochloric acid and extracted with ethyl acetate. The organic phase was dried, and the residue was applied to a silicic acid column; PGB was eluted by a mixture of benzene-ethyl acetate (60:40) according to the method of Jaffe, Behman, and Parker [32]. The PGB fraction was dried and measured radioimmunologically using PGB antiserum (CA 501, Clinical Assay), which does not distinguish $PGB_1$ from $PGB_2$. The overall recovery rate of added PGB (1 to 3 ng) was 54.8 ± 0.7% (mean ± SEM, $N = 15$). The estimated value was corrected for this loss.

Urinary $PGF_{2\alpha}$ also was measured radioimmunologically. Urine (3 to 5 ml) was acidified to a pH of 3 to 4 with hydrochloric acid and extracted with ethyl acetate. The organic phase was dried, the residue was applied to a silicic acid column, and $PGF_{2\alpha}$ was eluted by mixture of benzene-ethylacetate-methanol (60:40:20). The $PGF_{2\alpha}$ fraction was dried and measured radioimmunologically using $PGF_{2\alpha}$ antiserum.

The major urinary metabolite of $PGF_{2\alpha}$ (5α, 7α-dihydroxy-11-keto tetranor prostan-1,16-dioic acid) was measured by the method of Ohki, Hanyu, and Imaki [33]. Diluted urine (corresponding to 0.01 to 0.05 ml of original urine) was directly measured radioimmunologically using $PGF_{2\alpha}$-MUM antiserum and the $PGF_{2\alpha}$ urinary metabolite $^{125}$I-tyrosine methyl ester amide. This antiserum did not cross-react with the principal $PGE_1$ urinary metabolites 15-keto $PGF_{2\alpha}$, 15-keto $PGE_1$, and 15-keto $PGE_2$.

*Plasma renin activity.* PRA was determined by radioimmunoassay of angiotensin I (AI) [34]. Plasma, 1.0 ml, was adjusted to a pH of 5.5 and incubated at 37° C for 6 hours with EDTA and diisopropyl fluorophosphate (DFP). After the incubation, the sample was diluted tenfold with physiologic saline and heated in a boiling water bath for 15 min. After centrifugation, AI in the supernatant was

assayed radioimmunologically. PRA was expressed in terms of nanograms of generated AI per milliliter of plasma per hour of incubation. This method was approximately four times more sensitive than the method of Haber et al [35].

*Plasma aldosterone concentration.* PAC was measured with a commercial radioimmunoassay kit (Cer Ire Sorin). This method is sensitive down to 10 pg of aldosterone.

*Electrolytes.* Serum and urinary sodium and potassium were measured with an autoanalyzer. All results were expressed as the means ± SEM. The significance of differences was evaluated by Student's *t* test.

*Ad lib sodium diet.* Eighty-four healthy subjects (61 men and 23 women; 40.0 ± 1.6 years) were allowed to be on an unrestricted sodium diet and were hospitalized during the study. Blood was sampled in the morning after an overnight fasting, and 24-hr urine samples were collected in a bottle kept in a refrigerator.

*Circadian variation.* Healthy male physicians, aged 30 to 46 years, were included in this study. They were allowed to take an unrestricted diet. The subjects kept a supine position in a bed throughout the study except during urination, defecation, and meals. The study commenced at 9:00 A.M. All subjects emptied their bladders at this time, and the specimens were discarded. Urine was collected every 3 hours, and venous blood was sampled every 6 hours.

*Influence of low sodium diet and high sodium diet.* Advanced cases of hypertension, 5 men and 2 women, ranging in age from 23 to 51 years, hospitalized in our clinic with the diagnosis of essential hypertension (6 cases) and renovascular hypertension (1 case), were studied. Antihypertensive medication was discontinued at least for 2 weeks. They were given a diet containing 200 mEq of sodium daily for a week and then 30 mEq of sodium for 5 days, 350 mEq of sodium for 5 days, 350 mEq of sodium with 96 mEq of potassium for 3 days, and finally 350 mEq of sodium with unrestricted potassium diet for 3 days. Twenty-four hour urine samples were collected during each period.

*Influence of acute water loading.* Eight hospitalized hypertensive patients (4 men and 4 women; 25 to 57 years), including 7 cases of essential hypertension and 1 case of renovascular hypertension, were subjected to this study. They were given an unrestricted diet. After the collection of 24-hour urine samples ($U_1$), the patients were kept supine for 2 hours in the next morning, and urine was collected during this period as a control ($U_2$). Then, water (20 ml/kg) was ingested over 45 min, and urine was collected serially at the end of the loading ($U_3$) and every 30 min thereafter for 2.5 hours ($U_4$ to $U_8$). Blood samples were taken before and 75, 135, and 195 min after the water loading.

*Influence of acute saline infusion.* Twenty hospitalized hypertensive patients (11 men and 9 women, 21 to 53 years) were studied. They were given an unrestricted diet. After overnight fasting, the subjects were kept in a supine position for 2 hours. Urine and blood samples were obtained, and then physiologic saline was infused i.v. at a constant rate of 10 ml/min for 100 min. After the infusion, urine and blood samples were again collected.

*Furosemide administration and upright posture.* Sixteen healthy volunteers (12 men and 4 women, 27.7 ± 2.3 years) were included in this study. The subjects were allowed to take an unrestricted diet. After overnight fasting, the subjects were kept in a supine position for at least 1 hour, and then furosemide (1 mg/kg) was injected into the antecubital vein. The subjects remained in an upright posture for 120 min thereafter. Urine and blood samples were obtained before and 30 and 120 min after the furosemide administration.

*Administration of spironolactone.* Three patients with essential hypertension were allowed to take an unrestricted diet for 2 weeks, and then spironolactone (100 mg/day) was given for a week. Samples of blood and urine were taken before and after the spironolactone administration.

*Administration of indomethacin.* Eleven hypertensive patients were given a diet containing 90 mEq/day of sodium. Furosemide (80 mg/day) was given orally for 3 days, then indomethacin (150 mg/day, orally) was added for an additional 3 days. Samples of blood and urine were taken before and at the end of each 3-day period.

In these experiments, the urine and the plasma were stored at −20° C for the later determination of kallikrein, kinin, PGE, PRA, and PAC.

## Results

*Urinary excretion of kallikrein during ad lib sodium diet.* The basal level of urine flow, the urinary excretion of sodium, potassium, kallikrein, and PGE, PRA, and PAC in 84 healthy subjects eating an unrestricted diet are shown in Table 1. Among these parameters, there were significant correlations between the urinary excretion of kallikrein and the urine flow ($r = 0.30$), and between urinary excretion of kallikrein and that of potassium ($r =$

**Table 1.** Characteristics of normal subjects on an ad lib sodium diet

| | |
|---|---|
| No. of subjects | 84 |
| Age, *years* | 40.0 ± 1.6 |
| Mean arterial pressure, *mm Hg* | 94 ± 1 |
| Plasma renin activity, *ng/ml/hr* | 1.5 ± 0.2 |
| Plasma aldosterone concentration, *ng/dl* | 6.2 ± 0.5 |
| Urinary volume, *ml/day* | 1878 ± 67 |
| Urinary sodium excretion, *mEq/day* | 281 ± 11 |
| Urinary potassium excretion, *mEq/day* | 52 ± 3 |
| Urinary prostaglandin E excretion, *ng/day* | 736 ± 32 |
| Urinary kallikrein excretion, *μg/day* | 34.5 ± 4.0 |

0.36). There were also significant correlations between urinary excretion of PGE and that of sodium ($r = 0.39$), between PGE and potassium ($r = 0.33$), and between PGE and urine flow ($r = 0.30$). On the contrary, no significant correlation was found between urinary excretion of kallikrein and that of sodium, between kallikrein and PGE, between kallikrein and PRA, between urinary excretion of kallikrein and PAC, between urinary excretion of PGE and PRA, and between urinary excretion of PGE and PAC.

*Circadian variation.* Eight of ten subjects showed a prominent circadian variation in each parameter. Figure 1 demonstrates the circadian variation of urine flow, urinary sodium excretion, urinary potassium excretion, urinary PGE excretion, urinary kallikrein excretion, urinary kinin excretion, PRA, and PAC in 10 healthy volunteers.

Urine flow fell to a minimum of 46 ± 7 ml/hr between 3:00 and 6:00 A.M. and then rose to a maximum of 112 ± 15 ml/hr between 12:00 noon and 3:00 P.M. This represented a 71% variation for the 24-hr mean level. Urinary excretion of sodium fell to minimum of 5.1 ± 0.8 mEq/hr between 3:00 and 6:00 A.M. and rose to a maximum of 15.0 ± 1.5 mEq/hr between 12:00 noon and 3:00 P.M. This was a 92% variation for the 24-hr mean level. Urinary excretion of potassium fell to a minimum of 1.0 ± 0.1 mEq/hr between midnight and 3:00 A.M. and rose to a maximum of 3.1 ± 0.4 mEq/hr between 9:00 A.M. and 12:00 noon. This was a 102% variation for the 24-hr mean level. Urinary potassium excretion changed 3 hours earlier than the other parameters. Urinary excretion of kallikrein rose to a maximum of 2.57 ± 0.49 μg/hr between 6:00 and 9:00 A.M. and then fell to a minimum of 1.22 ± 0.33 μg/hr between 3:00 and 6:00 P.M. This was an 84% variation for the 24-hr mean level. Urinary excretion of kinin rose to a maximum of 870 ± 156 ng/hr between 12:00 noon and 3:00 P.M. and then fell to a minimum of 418 ± 35 ng/hr between 9:00 P.M. and

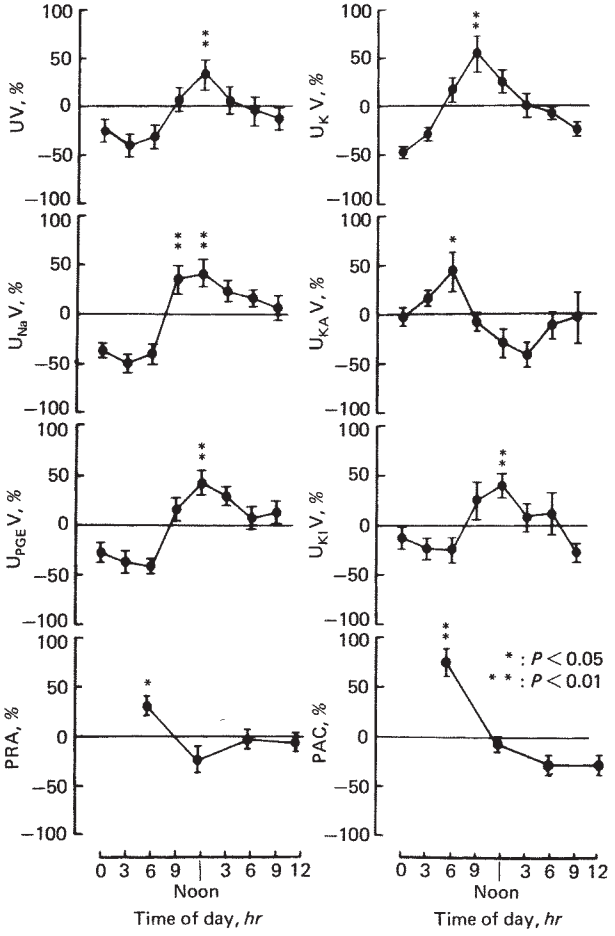


**Fig. 1.** *Circadian variation of urinary volume (UV), urinary sodium excretion ($U_{Na}V$), urinary potassium excretion ($U_KV$), urinary prostaglandin E excretion ($U_{PGE}V$), urinary kallikrein excretion ($U_{KA}V$), urinary kinin excretion ($U_{KI}V$), plasma renin activity (PRA), and plasma aldosterone concentration.* Variation is expressed as a shift from 24-hour mean level of each parameter.

midnight. This was a 64% variation for the 24-hr mean level. Urinary excretion of PGE fell to minimum of 12.0 ± 1.3 ng/hr between 6:00 and 9:00 A.M. and then rose to a maximum of 30.0 ± 3 ng/hr between 12:00 noon and 3:00 P.M. This was an 83% variation for the 24-hr mean level. A reversed relation of circadian variation was found between urinary kinin and urinary kallikrein. Urinary kinin excretion followed that of kallikrein, with a delay of 6 hours. On the other hand, PRA and PAC rose to maximum values of 1.97 ± 0.38 ng/ml/hr and 8.4 ± 1.7 ng/dl at 6:00 P.M. and then fell to minimum values of 1.1 ± 0.1 ng/ml/hr at 12:00 noon and of 2.9 ± 1.3 ng/dl at 6:00 P.M., with 53% and 122% variations for the 24-hr mean levels, respectively. Average urinary kinin excretion was significantly positively correlated with average urine flow ($r =$

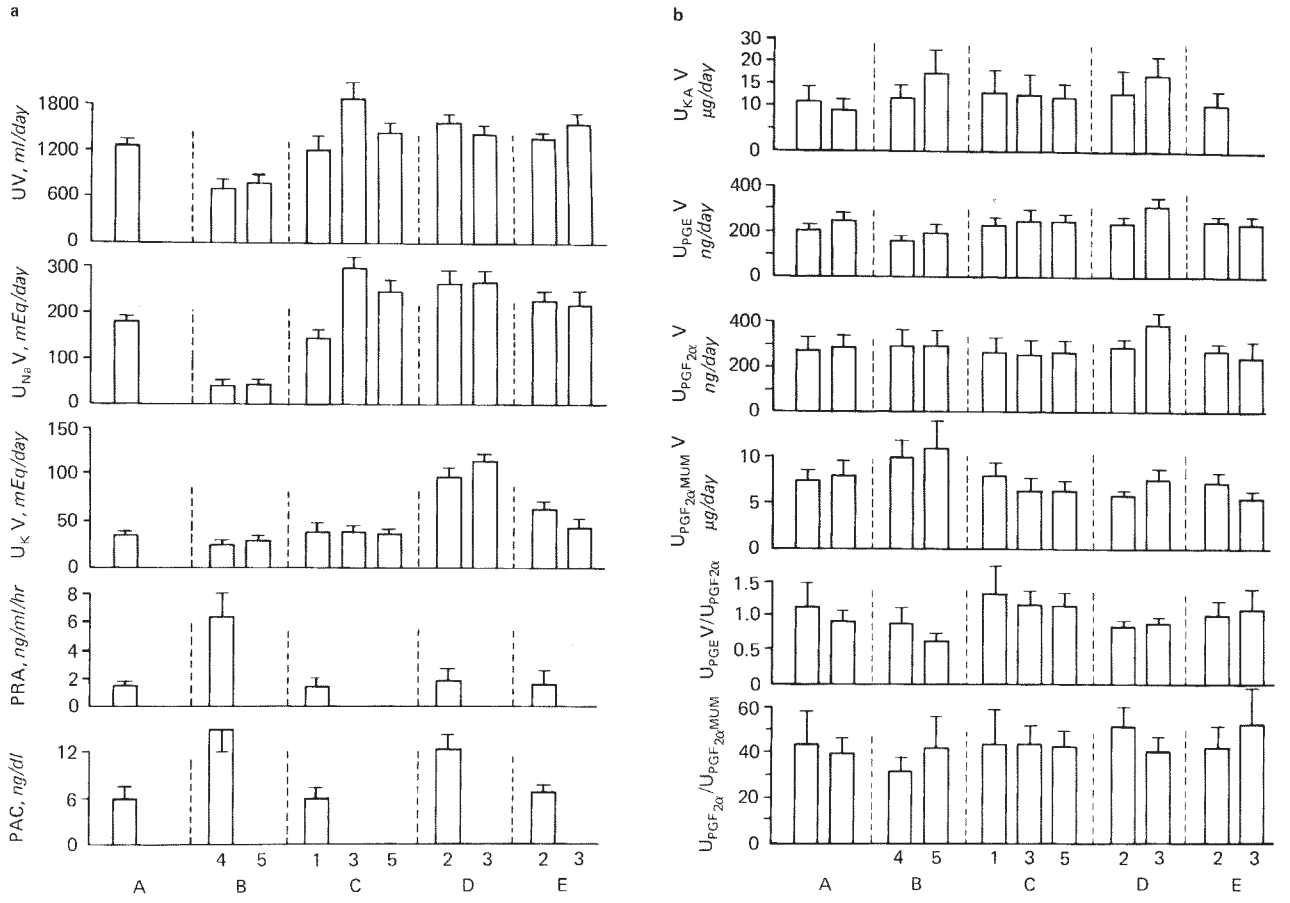


**Fig. 2.** *Influence of low sodium and high sodium diet on urinary volume (UV), urinary sodium excretion ($U_{Na}V$), urinary potassium excretion ($U_KV$), urinary kallikrein excretion ($U_{KA}V$), urinary prostaglandin E ($U_{PGE}V$) and $F_{2\alpha}$ ($U_{PGF_{2\alpha}}V$) excretion, urinary excretion of main urinary metabolite of $PGF_{2\alpha}$ ($PGF_{2\alpha\text{-}MUM}V$), the ratio of urinary PGE excretion to urinary $PGF_{2\alpha}$ excretion to urinary $PGF_{2\alpha}$ main urinary metabolite excretion ($U_{PGF2\alpha}V/U_{PGF2\alpha\text{-}MUM}V$).* A denotes the first period (7 days), when a diet containing 200 mEq/day of sodium was given. B denotes the second period (5 days) when a diet containing 30 mEq/day of sodium was given. C is the third period (5 days) when 350 mEq/day of sodium was given. D is the fourth period (3 days) when 350 mEq/day of sodium and 96 mEq/day of potassium were given. E is the last period (3 days) when 350 mEq/day of sodium with unrestricted potassium diet was given. The numbers in each period express the days of blood and urine sampling.

0.90, $P < 0.01$), average sodium excretion ($r = 0.83$, $P < 0.01$), and average urinary PGE excretion ($r = 0.76$, $P < 0.05$). In contrast with urinary kinin, average urinary kallikrein excretion was significantly negatively correlated with average urine flow ($r = -0.83$, $P < 0.01$), average sodium excretion ($r = -0.81$, $P < 0.01$), and average urinary PGE excretion ($r = -0.88$, $P < 0.01$). There was a significant positive correlation between PAC and urinary kallikrein excretion in each case ($r = 0.47$, $P < 0.05$). No significant correlation was found between PRA and urinary kallikrein excretion or urinary kinin excretion in each case. For the relation between urinary PGE and the renin-angiotensin-aldosterone system, there was a significant positive correlation between urinary PGE and PAC in each ($r = 0.56$, $P < 0.01$), whereas a significant negative correlation between PRA and urinary PGE excretion ($r = -0.78$, $P < 0.01$) was noted. There was also a positive correlation between average urinary PGE excretion and average urinary sodium excretion ($r = 0.96$, $P < 0.01$), or urine flow ($r = 0.93$, $P < 0.01$).

*Influence of low sodium diet, high sodium diet, and high potassium intake.* The changes of urine flow, PRA, PAC, and urinary excretion of sodium, potassium, kallikrein, PGE, $PGF_{2\alpha}$, and the main urinary metabolite of $PGF_{2\alpha}$ following the addition of a diet containing 200 mEq of sodium, 30 mEq of sodium, 350 mEq of sodium, 350 mEq of sodium with 96 mEq of potassium chloride are illustrated in Fig. 2, a and b. During sodium depletion, urinary excretion of kallikrein and $PGF_{2\alpha}$ tended to increase, and there was a prominent augmentation of PRA and PAC, whereas urinary PGE excretion

tended to decrease. During high sodium intake, PRA, PAC, and the urinary $PGF_{2\alpha}$ metabolite were significantly decreased. The urinary excretion of kallikrein tended to decrease and the urinary excretion of PGE tended to increase, but these changes were not significant. Potassium loading induced a significant increase in PAC, urinary PGE excretion, and urinary $PGF_{2\alpha}$ excretion. The urinary excretion of kallikrein and $PGF_{2\alpha}$ metabolite also tended to increase after the potassium loading but these changes were not significant. There were significant positive correlations between urinary excretion of kallikrein and that of potassium ($r = 0.35$, $P < 0.05$), and between kallikrein and PGE ($r = 0.54$, $P < 0.01$). No significant correlation was found, however, between urinary kallikrein excretion and urine flow, or urinary sodium excretion. On the other hand, there were significant correlations between urinary excretion of PGE and urine flow ($r = 0.54$, $P < 0.01$), between PGE and sodium ($r = 0.57$, $P < 0.01$), and between PGE and potassium ($r = 0.54$, $P < 0.01$). No significant correlation was found between urinary kallikrein excretion and PRA, or PAC. There was also no significant correlation between urinary PGE excretion and PRA or PAC.

*Influence of acute water loading.* Changes of urine flow, urinary excretion of potassium, sodium, kallikrein, kinin, and PGE, PRA, and PAC following acute water loading are illustrated in Fig. 3. Urine flow, urinary kallikrein excretion, urinary kinin excretion, and urinary PGE excretion were increased rapidly after water loading, reached a maximum in 45 min ($U_4$), and remained at this level for 90 min. Then the excretion rate fell, returning to the control level after 165 min ($U_8$). In contrast to the urinary kallikrein-kinin and PGE, the PRA and PAC did not change significantly. The urinary excretion of potassium and sodium tended to increase after the water loading but not significantly. There were significantly positive correlations between urinary kinin excretion and urinary kallikrein excretion. Significantly positive correlations were also found between the urinary excretion of PGE and that of kinin or kallikrein. Urine flow was highly correlated with these three parameters, urinary kinin, urinary kallikrein, and urinary PGE.

*Influence of acute saline infusion.* The changes of urine flow, the fractional excretion of sodium and potassium, the urinary excretion of PGE, kinin, and kallikrein, and the PRA and PAC before and after saline loading are illustrated in Fig. 4. A significant increase in urine flow, fractional excretion of sodium and urinary excretion of PGE, kallikrein, and

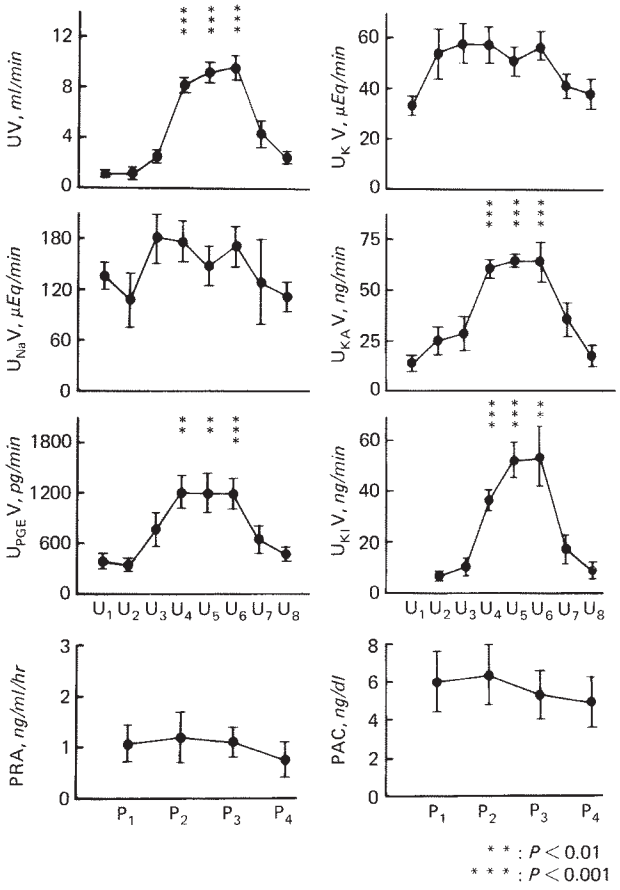


**Fig. 3.** *Influence of acute water loading on urinary volume (UV), urinary sodium excretion ($U_{Na}V$), urinary potassium excretion ($U_KV$), urinary prostaglandin excretion ($U_{PGE}V$), urinary kallikrein excretion ($U_{KA}V$), urinary kinin excretion ($U_{KI}V$), plasma renin activity (PRA), and plasma aldosterone concentration (PAC).* Explanation of abbreviations ($U_{1-8}$, $P_{1-4}$) is given in the text.

kinin were found after the acute saline infusion despite a decrement of PRA and PAC. There were significantly positive correlations between urinary excretion of kinin and kallikrein, between kinin and PGE, and between kallikrein and PGE. The urine flow and the fractional excretion of sodium were highly correlated with the urinary excretion of kinin, kallikrein, and PGE. In contrast, a negative correlation was found between PAC and urinary kinin, or kallikrein, and between PRA and urinary kinin, or kallikrein.

*Effect of furosemide and upright posture.* Changes of urine flow, urinary excretion of kinin, kallikrein, PGE, $PGF_{2\alpha}$, and $PGF_{2\alpha}$ metabolite, and PRA and PAC following the i.v. administration of furosemide and assumption of upright posture are shown in Table 2. Urine flow and urinary excretion of sodium, potassium, kinin, kallikrein, PGE, and

**Table 2.** Effects of furosemide and upright posture on each parameter in 16 normal subjects[a]

| | Control | Furosemide | |
|---|---|---|---|
| | | After 30 min | After 120 min |
| UV, *ml/min* | 1.0 ± 0.1 | 14.6 ± 1.1[d] | 6.6 ± 0.6[d] |
| $U_{Na}V$, *mEq/min* | 169 ± 16 | 1928 ± 135[d] | 812 ± 93[d] |
| $U_KV$, *mEq/min* | 39 ± 5 | 136 ± 10[d] | 90 ± 8[b] |
| PRA, *ng/ml/hr* | 1.9 ± 0.5 | 5.8 ± 0.8[d] | 8.6 ± 0.7[d] |
| PAC, *ng/dl* | 4.3 ± 0.5 | 8.2 ± 1.1[c] | 15.9 ± 2.4[d] |
| $U_{PGE}V$, *ng/min* | 0.49 ± 0.07 | 1.30 ± 0.18[b] | 0.52 ± 0.05 |
| $U_{PGF_{2\alpha}}V$, *ng/min* | 0.32 ± 0.02 | 0.96 ± 0.21[b] | 0.50 ± 0.07 |
| $U_{PGF_{2\alpha}\text{-MUM}}V$, *ng/min* | 17.1 ± 2.9 | 10.7 ± 1.3[b] | 8.2 ± 0.9[c] |
| $U_{KA}V$, *ng/min* | 44.9 ± 6.7 | 143.0 ± 21.4[c] | 70.4 ± 15.3 |
| $U_{KI}V$, *ng/min* | 10.5 ± 1.9 | 40.6 ± 8.6[c] | 23.0 ± 5.4[b] |

[a] Values are the means ± SEM. Abbreviations used are UV, urinary volume; $U_{Na}V$, urinary sodium excretion; $U_KV$, urinary potassium excretion; PRA, plasma renin activity; PAC, plasma aldosterone concentration; $U_{PGE}V$, urinary prostaglandin E excretion; $U_{PGF_{2\alpha}}V$, urinary prostaglandin $F_{2\alpha}$ excretion, $U_{PGF_{2\alpha}\text{-MUM}}V$, urinary excretion of the main urinary metabolite of prostaglandin $F_{2\alpha}$; $U_{KA}V$, urinary kallikrein excretion; $U_{KI}V$, urinary kinin excretion.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

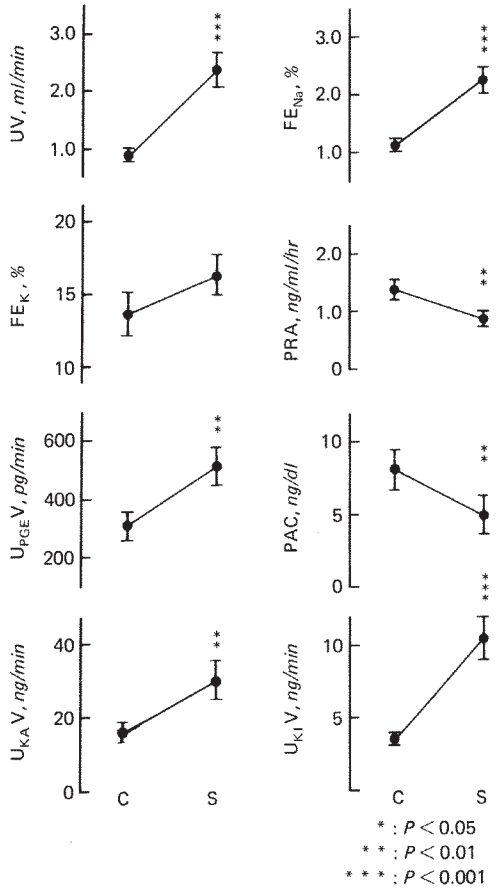


**Fig. 4.** *Influence of acute saline infusion on urinary volume (UV), fractional sodium excretion ($FE_{Na}$), fractional potassium excretion ($FE_K$), urinary prostaglandin E excretion ($U_{PGE}V$), urinary kallikrein excretion ($U_{KA}V$), urinary kinin excretion ($U_{KI}V$), plasma renin activity (PRA), and plasma aldosterone concentration (PAC).* Abbreviations are C, control period; S, after saline infusion.

$PGF_{2\alpha}$ were prominently increased within the first 30 min after the furosemide injection and then returned to the control level at 120 min. In contrast to urinary PGE and $PGF_{2\alpha}$, the metabolite of urinary $PGF_{2\alpha}$ was continuously decreased after furosemide administration. On the other hand, PRA and PAC were continuously increased for 2 hours after the injection. The time courses of the increase in PRA and PAC were different from those of urinary kinin, urinary kallikrein, and urinary PGE, urine flow, and urinary potassium and sodium concentrations. In comparison with the early increase in urinary kinin, kallikrein and PGE, the increases in PRA and PAC were delayed. There were significantly positive correlations between the urinary excretion of kinin and that of kallikrein, between kinin and PGE, and between kallikrein and PGE. Highly significant correlations were also found between the urinary excretion of sodium and that of kinin or kallikrein. In contrast, no significant correlation was found between PRA and the urinary excretion of kinin, or that of kallikrein, and between PAC and the urinary excretion of kinin or that of kallikrein.

*Effect of spironolactone.* The changes in the urinary excretion of sodium, kallikrein, and PGE following spironolactone administration in 3 patients with essential hypertension are shown in Table 3. Urinary kallikrein excretion gradually decreased during spironolactone administration, but the urinary excretion of sodium and PGE increased immediately after spironolactone administration began and then decreased gradually to the control level.

**Table 3.** Effects of spironolactone on each parameter in 3 patients with essental hypertension[a]

| | Case 1 | | | Case 2 | | | Case 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $U_{Na}V$ *mEq/day* | $U_{PGE}V$ *ng/day* | $U_{KA}V$ *μg/day* | $U_{NA}V$ *mEq/day* | $U_{PGE}$ *ng/day* | $U_{KA}V$ *μg/day* | $U_{Na}V$ *mEq/day* | $U_{PGE}V$ *ng/day* | $U_{KA}V$ *μg/day* |
| Control period | 271 | 265 | 57 | 301 | 182 | 8.3 | 149 | 151 | 44 |
| After spironolactone administration | | | | | | | | | |
| Day 1 | 497 | 670 | 37 | 357 | 293 | 9.0 | 207 | 402 | 40 |
| Day 2 to 3 | 349 | 251 | 42 | 214 | 130 | 2.7 | 207 | 265 | 30 |
| Day 4 to 5 | 235 | 187 | 19 | 157 | 255 | 2.9 | 147 | 114 | 30 |
| Day 6 to 7 | 283 | 128 | 20 | 217 | 290 | 5.0 | 66 | 189 | 20 |

[a] Values are presented as an actual value or the mean value in each period. Abbreviations are defined in Table 2.

**Table 4.** Effects of low sodium diet with an oral administration of furosemide and the additional effect of indomethacin on each parameter in 11 patients with essential hypertension[a]

| | Control | Low Na diet + furosemide | Low Na diet + furosemide + indomethacin |
|---|---|---|---|
| $U_{Na}V$, *mEq/day* | 183 ± 22 | 84 ± 8[d] | 64 ± 7 |
| PRA, *ng/ml/hr* | 1.2 ± 0.2 | 5.5 ± 1.7[b] | 1.6 ± 0.2 |
| PAC, *ng/dl* | 5.9 ± 1.2 | 19.1 ± 3.9[c] | 4.8 ± 1.2 |
| $U_{PGE}V$, *ng/day* | 298 ± 62 | 330 ± 59 | 170 ± 33[b] |
| $U_{KA}V$, *μg/day* | 22.4 ± 8.6 | 37.1 ± 16.7 | 24.5 ± 8.6 |

[a] Abbreviations are defined in Table 2.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

*Effect of indomethacin.* The changes in the urinary excretion of sodium, kallikrein, and PGE, and in PRA and PAC before and after the administration of indomethacin to hypertensive patients are shown in Table 4. On a low sodium diet and after the oral administration of furosemide, PRA and PAC were prominently increased. Urinary kallikrein excretion tended to increase but not significantly. Urinary PGE excretion did not change. On the other hand, indomethacin induced a significant decrease in PRA, PAC, and urinary PGE excretion. Urinary excretion of sodium and kallikrein tended to decrease after indomethacin administration but not significantly.

## Discussion

The urinary kallikrein excretion was decreased after spironolactone administration in patients with hypertension, and the urinary kallikrein excretion was increased after stimulation of the renin-angiotensin-aldosterone system by furosemide injection accompanied by upright posture in healthy volunteers. But, there was no significant correlation between urinary kallikrein excretion and PAC after the furosemide administration, the time courses of changes in the two variables being quite different. In the present data, there was also no significant correlation between the urinary kallikrein excretion and the PAC before and after the administration of a low sodium diet, high sodium diet, and potassium chloride loading in hypertensive patients, although the urinary kallikrein tended to increase after the dietary sodium depletion and the potassium chloride loading, and tended to decrease after the dietary sodium repletion. The basal levels of urinary kallikrein excretion in 84 healthy subjects did not show any significant correlation with PAC levels. These data indicate that there may be another regulator of the release and/or the generation of renal kallikrein in addition to aldosterone.

It is well known that there is circadian rhythm in the secretion of aldosterone [36, 37]. In 1977, Bowden et al [38] reported that there was a circadian rhythm in the excretion of urinary PGE. Little is known, however, about the circadian variation for the urinary kallikrein excretion. The present study clearly indicates the presence of a circadian variation in the excretion of urinary kinin and kallikrein, urine flow, urinary sodium excretion, urinary potassium excretion and urinary PGE excretion. How does the circadian variation of the urinary excretion of kinin and kallikrein occur? In the present data, the pattern of urinary kinin excretion coincided with those of urine flow, urinary sodium excretion, and urinary PGE excretion. There was also a significant correlation between urinary kinin excretion and urine flow, urinary sodium excretion, and uri-

nary PGE excretion. These data indicate that the urinary excretion of kinin and PGE may depend on the rate of urine flow or urinary sodium excretion. In contrast to the urinary kinin excretion, significant negative correlations were found between the urinary kallikrein excretion and the urine flow, urinary sodium excretion, or urinary PGE excretion. The acrophase of urinary kallikrein excretion was 6 hours earlier than those of urine flow and urinary excretion of sodium, kinin, and PGE. These results indicate that the excretion of urinary kallikrein may be independent of urine flow and urinary sodium excretion.

A highly positive correlation was found in the circadian variations between urinary kallikrein and PAC, whereas negative correlations were found between PAC and urinary kinin excretion, or urinary PGE excretion. The acrophase of urinary kallikrein excretion was 1.5 hours later than that of PAC. These data suggest that the circadian variation of urinary kallikrein may be regulated by aldosterone. The nadir of urinary kallikrein excretion, however, was found between 3:00 and 6:00 P.M., and then kallikrein excretion increased gradually despite continuation of the low level of PAC until midnight. From these results, the regulatory mechanism of renal kallikrein between 3:00 P.M. and midnight could not be explained by aldosterone. In the present data, a mirror image was found between the circadian variation in urinary kinin and kallikrein, with a highly negative correlation being found between the two. These findings are in good accordance with the report of Vinci et al [39] that urinary kinin excretion was low in patients with Bartter's syndrome in the presence of an augmented urinary kallikrein excretion. How can the discrepancy in the urinary excretion of kallikrein and kinin be explained? The present data that the phase of the circadian variation of urinary kinin was delayed from that of urinary kallikrein and that the acrophase of urinary kallikrein coincided with the nadir of urinary kinin and the nadir of the former with the acrophase of the latter suggest that there may be a feedback mechanism in the renal kallikrein-kinin system by which kallikrein enhances the kinin generation and then, in turn, accumulated kinin inhibits the release or the generation of kallikrein. This feedback mechanism may contribute to the regulation of kallikrein, in addition to the regulatory effect of aldosterone. The present data that the circadian rhythm of urinary PGE excretion was synchronized with that of urinary kinin excretion suggest that renal kinin may contribute to the regulatory mechanism of circadian variation of urinary PGE. The synchronization of the circadian variations of kinin, PGE, urine flow, and urinary sodium indicates that renal kinin and PGE may be involved in the regulation of water and sodium excretion.

In this study, urinary excretion of kinin, kallikrein, and PGE was significantly increased after the acute expansion of the extracelllar fluid volume with isotonic saline despite inhibition of the renin-angiotensin-aldosterone system. Similar changes in urinary excretion of kinin, kallikrein, and PGE were also found after water loading. These results indicate that acute volume expansion stimulates the release and/or the generation of renal kallikrein-kinin and PGE independently of the renin-angiotensin-aldosterone system. But, it is very difficult to ascertain whether the augmented urinary kallikrein-kinin and PGE are the cause of diuresis and natriuresis after acute volume expansion or merely whether they reflect a washout phenomenon induced by the enhanced urine flow.

There are many reports [40–42] that the PG synthetase inhibitor indomethacin inhibits the diuresis and the enhancement of PRA and PAC induced by furosemide. The recent papers by Weber, Schere, and Larsson [43] and Gerber and Nies [44] showed that furosemide stimulates the release of free arachidonic acid, a main precursor of PG's, in man and in dog. In the present data, however, the urinary excretion of the $PGF_{2\alpha}$ metabolite was decreased after the i.v. administration of furosemide despite enhancement of urinary excretion of PGE and $PGF_{2\alpha}$. These data suggest that the augmentation of urinary PGE and $PGF_{2\alpha}$ excretion following furosemide administration might be due to the inhibition of PG metabolism, probably by the inhibition of 15-hydroxy prostaglandin dehydrogenase by this diuretic itself, as shown by Paulsrud and Miller [45], though furosemide also facilitates the generation of PG's. The present data that the time course of PRA alteration following furosemide administration was different from that of urinary PGE excretion indicate that the augmentation of urinary PGE excretion was independent of the renin-angiotensin system enhancement by furosemide. Our data that urinary excretion of kallikrein and kinin following furosemide administration was highly correlated with urinary PGE excretion support the presence of a linkage between the renal PGE system and the renal kallikrein-kinin system. This linkage was also found after acute volume expansion with saline or water.

The increased PRA induced by a low sodium diet and by furosemide administration was found to be suppressed by the addition of indomethacin. Urinary PGE excretion was not increased, however, after sodium deprivation despite the increase in PRA. These data indicate that renal PG, not PGE in the renal tubular compartment but probably $PGI_2$ in the vascular compartment, may contribute to the macula densa mechanism of renin release in man.

*Conclusion.* The present study demonstrates that there was a circadian variation in the excretion of urinary kinin, kallikrein, and PGE, and that the circadian variation of urinary kallikrein may be regulated by aldosterone, whereas those of urinary kinin and PGE may depend on the rate of urine flow or urinary sodium excretion. The reversed relation of circadian variation between urinary kinin and urinary kallikrein suggests that there may be a feedback mechanism in the renal kallikrein-kinin system. The urinary excretion of kinin, kallikrein, and PGE was significantly increased after acute volume expansion with isotonic saline and water loading suggesting that the urinary excretion of PGE, kallikrein, and kinin after acute volume expansion may be dependent on the rate of urine flow.

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## References

1. ABE K: Urinary excretion of kinin in man with special reference to its origin. *Tohoku J Exp Med* 87:175–184, 1965
2. NUSTAD K: Localization of kininogenase in the rat kidney. *Br J Pharmacol* 39:87–98, 1970
3. CARRETERO OA, SCICLI AG: Renal kallikrein: its localization and possible role in renal function. *Fed Proc* 35:194–198, 1976
4. ØRSTAVIK TB, NUSTAD K, BRANDTZAEG P, PIERCE JV: Cellular origin of urinary kallikrein. *J Histochem Cytochem* 24:1037–1039, 1976
5. LARSSON C, ÄNGGÅRD E: Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur J Pharmacol* 21:30–36, 1973
6. LEE JB, COVINO BG, TAKMAN BH, SMITH ER: Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. *Circ Res* 17:57–77, 1965
7. FERRERIA SH, VANE JR: Prostaglandins: Their disappearance from and release into the circulation. *Nature* 216:868–873, 1967
8. LARSSON C, ÄNGGÅRD E: Mass spectrometric determination of prostaglandin $E_2$, $F_{2\alpha}$, and $A_2$ in the cortex and medulla of the rabbit kidney. *J Pharm Pharmacol* 28:326–328, 1976
9. FRÖLICH JC, WILLIAMS WM, SWEETMAN BJ, SMIGEL M, NIES AS, CARR K, WATSON JT, OATES JA: Urinary prostaglandins: identification and origin. *J Clin Invest* 55:763–770, 1975
10. ERDÖS EG, YANG HYT: Kininase, in *Handbook of Experimental Pharmacology: Bradykinin, Kallidin and Kallikrein,* edited by ERDÖS EG, New York, Springer, 1970, Vol. 25, pp. 289–323
11. GELLER RG, MARGOLIUS HS, PISANO JJ, KEISER HR: Effects of mineralocorticoids, altered sodium intake and adrenalectomy on urinary kallikrein in rats. *Circ Res* 31:857–861, 1972
12. MARGOLIUS HS, CHAO J, KAIZU T: The effects of aldosterone and spironolactone on renal kallikrein. *Clin Sci Mol Med* 3 (Suppl):279s–282s, 1976
13. MILLS IH, MACFARLANE NAA, WARD PE, OBIKA LFD: The renal kallikrein-kinin system in the regulation of salt and water excretion. *Fed Proc* 35:181–188, 1976
14. MCGIFF JC, ITSKOVITZ HD, TERRAGNO NA: The actions of bradykinin and eledoisin in the canine isolated kidney: Relationships to prostaglandins. *Clin Sci Mol Med* 49:125–131, 1975
15. NASJLETTI A, MCGIFF JC, COLINA-CHOURIO J: Interrelations of the renal kallikrein-kinin system and renal prostaglandins in the conscious rat: Influence of mineralocorticoids. *Circ Res* 43:799–807, 1979
16. MCGIFF JC, CROWSHAW K, TERRAGNO NA, LONIGRO AJ: Release of a prostaglandin-like substances into renal venous blood in response to angiotensin II. *Circ Res* 26–27 (Suppl. 1):I-121–I-130, 1970
17. LARSSON C, WEBER P, ÄNGGÅRD E: Arachidonic acid increases and indomethacin decreases plasma renin activity in the rabbit. *Eur J Pharmacol* 28:391–394, 1974
18. WEBSTER ME, GILMORE JP: Influence of kallidin-10 on renal function. *Am J Physiol* 206:714–718, 1964
19. JOHNSTON HH, HERZOG JP, LAULER DP: Effect of prostaglandin $E_1$ on renal hemodynamics, sodium and water excretion. *Am J Physiol* 213:939–946, 1968
20. MARIN-GREZ M, COTTONE P, CARRETERO OA: Evidence for an involvement of kinins in regulation of sodium excretion. *Am J Physiol* 223:794–796, 1972
21. MARIN-GREZ M, OZA NB, CARRETERO OA: The involvement of urinary kallikrein in the renal escape from the sodium retaining effect of mineralocorticoids. *Henry Ford Hosp Med J* 21:85–90, 1973
22. GELLER RG, MARGOLIUS HS, PISANO JJ, KEISER HR: Effects of mineralocorticoids, altered sodium intake and adrenalectomy on urinary kallikrein in rats. *Circ Res* 31:857–861, 1972
23. JOHNSTON CI, MATTHEWS PG, DAX E: Renin-angiotensin and kallikrein-kinin systems in sodium homeostasis and hypertension in rats. *Clin Sci Mol Med* 51:283s–286s, 1976
24. MARGOLIUS HS, HORWITZ D, GELLER RG, ALEXANDER RW, GILL JR JR, PISANO JJ, KEISER HR: Urinary kallikrein excretion in normal man: Relationships to sodium intake and sodium-retaining steroids. *Circ Res* 35:812–819, 1974

25. ABE K, YASUJIMA M, SAKURAI Y, CHIBA S, ITO T, IMAI Y, SATO M, HARUYAMA T, OMATA K, GOTO T, SATO K, HIWATARI M, OTSUKA Y, YOSHINAGA K: The role of renal prostaglandin E and kallikrein in pathogenesis of essential hypertension. *Jap Circ J* 43:1105–1116, 1979
26. TOBIAN L, O'DONNELL M: Renal prostaglandins in relation to sodium regulation and hypertension. *Fed Proc* 35:2388–2392, 1976
27. YASUJIMA M, ABE K, CHIBA S, SATO M, IROKAWA N, SEINO M, IMAI Y, SAITO K, SAKURAI Y, ITO T, HARUYAMA T, RITSU K, YOSHINAGA K: Implication of renal prostaglandin E in urinary sodium excretion. *Jap Circ J* 42:565–569, 1978
28. CARRETERO OA, OZA NB, PIWONSKA A, OCHOLIK T, SCICLI AG: Measurement of urinary kallikrein activity by kinin radioimmunoassay. *Biochem Pharmacol* 25:2265–2270, 1976
29. ABE K, KATO H, SAKURAI Y, ITOH T, SAITO K, HARUYAMA T, OTSUKA Y, YOSHINAGA K: Estimation of urinary kininogenase activity using bovine serum low molecular weight kininogen, in *Kinins-II: Biochemistry, Pathophysiology, and Clinical Aspects,* edited by FUJII S, MORIYA H, SUZUKI T, New York, Plenum, 1979, Vol. A, pp. 105–114
30. ABE K, YASUJIMA M, CHIBA S, IROKAWA N, ITOH T, YOSHINAGA K, SAITO T: Effects of furosemide on urinary excretion of prostaglandin E in normal volunteers and patients with essential hypertension. *Prostaglandins* 14:513–521, 1977
31. ZUSMAN RM: Quantitative conversion of PGA or PGE to PGB. *Prostaglandins* 1:167–168, 1972
32. JAFFE BM, BEHMAN HR, PARKER LW: Radioimmunoassay measurement of prostaglandin E, A and F in human plasma. *J Clin Invest* 52:398–405, 1973
33. OHKI S, HANYU T, IMAKI K: Radioimmunoassays of prostaglandins $F_{2\alpha}$-main urinary metabolite with prostaglandin $^{125}$I-tyrosine methyl ester amide. *Prostaglandins* 6:137–148, 1974
34. ABE K, OTSUKA Y, SAITO T, CHIN BS, AOYAGI H, MIYAZAKI S, IROKAWA N, SEINO M, YOSHINAGA K: Measurement of plasma renin activity by angiotensin I radioimmunoassay: A modification of Haber's method. *Jap Circ J* 36:741–749, 1972
35. HABER E, KOERNER T, PAGE LB, PURNODE A: Application of a radioimmunoassay for angiotensin I to the physiologic measurement of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab* 29:1349–1955, 1969
36. WILLIAMS GH, CAIN JP, DLUHY RG, UNDERWOOD RH: Studies of the control of plasma aldosterone concentration in normal man: I. Response to posture, acute and chronic volume depletion, and sodium loading. *J Clin Invest* 51:1731–1742, 1972
37. KATZ FH, ROMFH P, SMITH JA: Episodic secretion of aldosterone in supine man: Relationship to cortisol. *J Clin Endocrinol Metab* 35:178–181, 1972
38. BOWDEN RE, WARE JH, DEMETS DL, KEISER HR: Urinary excretion of immunoreactive prostaglandin E: a circadian rhythm and the effect of posture. *Prostaglandins* 14:151–161, 1977
39. VINCI JM, GILL JR JR, BOWDEN RE, PISANO JJ, IZZO JL JR, RADFAR N, TAYLOR AA, ZUSMAN RM, BARTTER FC, KEISER HR: The kallikrein-kinin system in Bartter's syndrome and its response to prostaglandin synthetase inhibition. *J Clin Invest* 61:1671–1682, 1978
40. PATAK RV, MOOKERJEE BK, BENTZEL CJ, HYSERT PE, BABEU M, LEE JB: Antagonism of the effects of furosemide by indomethacin in normal and hypertensive man. *Prostaglandins* 10:649–659, 1975
41. ROMERO JC, DUNLAP CL, STRONG CG: The effects of indomethacin and other anti-inflammatory drugs on the renin-angiotensin system. *J Clin Invest* 58:282–290, 1976
42. TAN SY, MULROW PJ: Inhibition of the renin-aldosterone response to furosemide by indomethacin. *J Clin Endocrinol Metab* 45:174–176, 1977
43. WEBER PC, SCHERE B, LARSSON C: Increase of free arachidonic acid by furosemide in man as the cause of prostaglandin and renin release. *Eur J Pharmacol* 41:329–332, 1977
44. GERBER JG, NIES AS: Furosemide-induced renal vasodilation: The role of the release of arachidonic acid. *Abst 4th Int Prostaglandin Conf,* Washington, D.C., 1979, p. 40
45. PAULSRUD JR, MILLER ON: Inhibition of 15-OH prostaglandin dephydrogenase by several diuretic drugs (abstr). *Fed Proc* 33:590, 1974